The transport properties of mitochondria are such that net potassium flux across the inner membrane determines mitochondrial volume. It has been known that K⁺ uptake is mediated by diffusive leak driven by the high electrical membrane potential maintained by redox-driven, electrogenic proton ejection and that regulated K⁺ efflux is mediated by an 82-kDa inner membrane K⁺/H⁺ antiporter. There is also long-standing suggestive evidence for the existence of an inner membrane protein designed to catalyze electrophoretic K⁺ uptake into mitochondria. We report reconstitution of a highly purified inner membrane protein fraction from rat liver and beef heart mitochondria that catalyzes electrophoretic K⁺ flux in liposomes and channel activity in planar lipid bilayers. The unit conductance of the channel at saturating [K⁺] is about 30 pS. Reconstituted K⁺ flux is inhibited with high affinity by ATP and ADP in the presence of divalent cations and by glibenclamide in the absence of divalent cations. The mitochondrial ATP-dependent K⁺ channel is selective for K⁺, with a Kₘ of 32 mM, and does not transport Na⁺. K⁺ transport depends on voltage in a manner consistent with a channel activity that is not voltage-regulated. Thus, the mitochondrial ATP-dependent K⁺ channel exhibits properties that are remarkably similar to those of the ATP-dependent K⁺ channels of plasma membranes.

The primary cause of electrophoretic K⁺ uptake into mitochondria is diffusive K⁺ leak across the inner membrane. At the high values of ΔΨ required for oxidative phosphorylation, K⁺ leak is not only unavoidable, it is also exponential with voltage (1). Unregulated K⁺ leak at high potentials is sufficiently hazardous to volume homeostasis that it would seem imprudent for mitochondria to contain a specific K⁺ uniport mechanism. Nevertheless, reconstitution experiments from several laboratories (2-7) have suggested that mitochondria possess an inner membrane K⁺ channel.

Since electrophoretic K⁺ uptake uncouples oxidative phosphorylation, it follows that this channel must be highly regulated, and this regulation is just beginning to be understood. Applying the patch clamp technique to intact mitochondrial membranes, Inoue et al. (8) detected a K⁺ channel that was blocked by ATP and glibenclamide. In independent studies, we had observed an extra component of K⁺ uptake into intact mitochondria over and above what could be ascribed to leak and that this component was inhibited by ATP (7). We undertook to identify and purify this activity by reconstitution of membrane protein fractions into proteoliposomes.³

We report purification of an inner membrane protein fraction from rat liver and beef heart mitochondria that induces K⁺ ion conductance when reconstituted into liposomes or incorporated into planar lipid bilayers. The predominant protein in the fraction is 54 kDa; however, the identity of the K⁺ channel protein has not yet been established with certainty. Reconstitution studies demonstrate that the mitochondrial K⁺ channel transports K⁺ electrophoretically and does not transport Na⁺. In the presence of Mg²⁺, K⁺ flux is blocked by ATP (K, ~45 μM) and ADP (K, ~280 μM). In the absence of Mg²⁺, K⁺ flux is blocked by glibenclamide (K, ~50 nM). These features bear striking similarities to the ATP-dependent K⁺ channels found in plasma membranes of heart, skeletal muscle, pancreatic β cell, and the central nervous system (for reviews, see Refs. 9 and 10).

EXPERIMENTAL PROCEDURES

Preparations and Materials—SMPs were prepared from rat liver and beef heart mitochondria as previously described (11). Mitochondria from rat liver were prepared (12) and further purified by centrifugation on linear sucrose density gradient. The latter step proved to be necessary for removal of a protein that copurified with the K⁺ channel. When we obtained N-terminal sequence of the contaminant, it turned out to be calrelin from endoplasmic reticulum (13). PBFI was obtained from Molecular Probes. Electrophoresis grade chemicals were purchased from Sigma.

Solubilization and Fractionation of the Mitochondrial K⁺ Channel—SMPs were solubilized at 6 mg of protein per ml in 3% Triton X-100, 20% glycerol, 0.1% β-mercaptoethanol, 0.2 mM EGTA, 1 mM MgCl₂, and 50 mM Tris-HCl, pH 7.2. After incubation on ice for 20 min, the mixture was centrifuged at 120,000 × g for 35 min. 10 ml of the supernatant containing 50-80 mg of extracted proteins was loaded

³ Preliminary reports of these findings have been presented (5-7).
onto a DEAE-cellulose column (10-ml bed volume) that had been equilibrated with a buffer containing 1% Triton X-100, 0.1% β-mercaptoethanol, 1 mM EDTA, and 50 mM Tris-HCl, pH 7.2. We first examined elution conditions using two bed volumes each of equilibration buffer to which was added 0, 50, 100, 200, 250, 300, and 500 mM KCl. Following this crude KCl step elution, we found K⁺ uniport activity solely in the 250 mM KCl fraction. Having identified the elution conditions, we were able to obtain a high degree of protein purification by a slight modification of the above protocol. As above, 10 ml of the supernatant containing 50–80 mg of extracted proteins was loaded onto a DEAE-cellulose column (30 ml bed volume) that had been equilibrated with equilibration buffer. The column was washed sequentially with 100 ml of equilibration buffer, then 60 ml each of buffer containing 100, 150, and 200 mM KCl. Finally, [KCl] was raised to 250 mM, and all of the K⁺ uniport activity was found within one bed volume (10 ml) of the eluate.

Eluted fractions were dialyzed against the equilibration buffer for 3–6 h and concentrated by filtration on an Amicon Centricon 30 microconcentrator. Proteins in fractions were analyzed by SDS-PAGE and for transport activity following reconstitution into liposomes.

**Reconstitution of Membrane Protein Fractions**—Reconstitution experiments were performed as previously described (14). Briefly, a 10:1 mixture of soybean α-lecithin and cardiolipin in chloroform was dried under nitrogen and ether and repurified in TEA⁻ salts, using the method of Bligh and Dyer (15), to remove Na⁺ and K⁺ ions. Purified lipids were dissolved in chloroform, dried under nitrogen, and stored over calcium hydroxide. The dried lipids were solubilized at 50 mg of lipid/ml in 0.8 ml of internal medium (defined below) containing 10% octylpentaoxyethylene, 300 μM PBFI, and 200 μl of the protein sample. This mixture was loaded onto a 2-ml Bio-beads SM-2 column, which had been pre-equilibrated with internal medium, to remove detergent and form proteoliposomes. After incubation for 90 min at 0–4 °C, the column was centrifuged at 4000 X g for 2 min to collect the proteoliposomes. To remove extravesicular PBFI, 300–μl aliquots of the proteoliposome suspension were passed twice through 1-ml Sephadex G-25–300 columns, which had been pre-equilibrated with internal medium lacking probe. Intraliposomal volume of each preparation was estimated from the volume of distribution of PBFI and was normally found to be 1 μl per mg of starting lipid. For these experiments, proteoliposomes were suspended in 150 mM KCl at 0.4 mg of lipid/ml, lysed, and starting PBFI was determined by standards addition of PBFI in 2 μl aliquots. Protein concentrations were estimated by the Biuret (16) and Amido Black methods (17). Bovine serum albumin was used as standard.

**Fluorescence Measurements**—The fluorescence emission intensity of PBFI is enhanced in the presence of K⁺. Fluorescence of probe-loaded proteoliposomes was measured with an SLM 8000 Fluorometer (SLM, Urbana, IL) connected to an IBM PS/2 Model 60 computer and calibrated as previously described (11, 14), except that excitation was set at 343 nm (slit width, 4 nm) and emission was set at 485 nm (slit width, 4 nm).

Two protocols were used for measurement of the electrophoretic K⁺ uptake as follows. (a) Vesicles (0.4 mg of lipid/ml) were added to a medium containing 150 mM KCl, 25 mM TEA-HEPES, pH 8.5, and 0.5 mM EDTA. Internal medium contained 0.10 mM KCl, 0.5 mM EDTA, 25 mM TEA-HEPES, pH 6.5, 100 mM TEA-SO₄, and 300 mM PBFI. The pH gradient (2 units) in the presence of 1 μM CCCP provided the electrical driving force for K⁺ uniport, and CCCP transport provided charge compensation. (b) Vesicles (0.4 mg of lipid/ml) were added to a medium containing 150 mM KSCN, 25 mM TEA-HEPES, pH 7.4, and 0.5 mM EDTA. Internal medium contained 0.10 mM KSCN, 0.5 mM EDTA, 25 mM TEA-HEPES, pH 7.4, 100 mM TEA-SO₄, and 300 mM PBFI. The permeable SCN⁻ anion clamped ΔΨ and provided charge compensation for electrophoretic K⁺ influx.

For calibration, proteoliposomes were treated with 0.5 μM nigericin and 5.0 μM tributyltin chloride to achieve [K⁺] equilibrium, followed by stepwise additions of aliquots of KCl (14).

**Electrical Measurements in Lipid Bilayer Membranes**—Lipids were isolated from bovine brain (18), solubilized in n-decane (20 mg/ml), and used to form a lipid bilayer membrane across a circular hole (0.975 × 10⁻⁴ cm²) in a Teflon partition separating two cuvettes (19). Medium on both sides of the membrane contained 1 mM KCl and 10 mM Tris-HCl, pH 7.2. The purified fraction, obtained as described above, was supplemented with phospholipid (0.5 mg/ml) and passed over a Bio-Bead SM-2 column to remove Triton. Aliquots of the eluate were added to the "cis" side of the membrane. Membrane conductivity was determined by the voltage-clamp method. Current across the membrane was measured with an electrometer amplifier and recorded.

**Electrophoresis—SDS-PAGE** was carried out according to Laemmli (20) using 7.5% polyacrylamide gels. Gel patterns were visualized with Coomassie Brilliant Blue R-250 and silver staining (21).

**RESULTS**

**Partial Purification of the Mitochondrial K⁺ Channel**—The detergent-solubilized extract of rat liver mitochondria was fractionated on a DEAE-cellulose column and assayed for K⁺ uniport activity following reconstitution, as described under "Experimental Procedures." The gel pattern following SDS-PAGE of fractions obtained using the crude KCl step gradient is shown in Fig. 1A; the sole fraction that exhibited activity is shown in lane 9.

The gel pattern of the 250 mM KCl fraction obtained after extensive washing with lower ionic strength buffers is contained in Fig. 1B. As can be seen in lane 3 of Fig. 1B, most of the contaminating proteins were removed, leaving a major band at 54 kDa. The same result was obtained when beef heart SMPs were purified with this protocol (not shown).

Silver staining following two-dimensional gel electrophoresis revealed a single protein at 54 kDa that migrated with pI ~6.8–7.2 (not shown). Silver staining of the SDS-PAGE gel also revealed several minor bands. This communication describes the transport properties of the mitochondrial K⁺ channel.
ductivity patterns were consistent with incorporation of mul-
planar lipid bilayer membranes. In most experiments, con-
dria induced large increases in the membrane conductivity of
tiple channels (not shown), but in some experiments the
situated with the partially purified fraction shown in lane
Fig. 1B.

Channel Properties of the Purified Protein Fraction—The
recording in Fig. 2 demonstrates that the purified fraction contains proteins that exhibit channel properties. Further
conductance studies will be reported in a separate publication.
The purified fractions from rat liver or beef heart mitochondria induced large increases in the membrane conductivity of

conductance studies will be reported in a separate publication. The purified fractions from rat liver or beef heart mitochon-
der such experiment is illustrated in Fig. 2, which contains a
recording of putative single channel events from the beef
heart protein. The calculated conductance in 1 M KCl was 30
pS. Considering that the $K_m$ for K$^+$ is about 30 mM (see
below), this value is in good agreement with previous results
from Mironova et al. (2) showing single channel conductance
of 1 pg per ml of the partially purified fraction to the
chamber separated by a planar lipid bilayer. A voltage of
applied across the membrane to obtain the current recording. The
observed upward deflection of the signal reflects opening of a channel
carrying a current of about 2.55 pA.

Reconstitution of Electrophoretic K$^+$ Flux—The traces in
Fig. 3 demonstrate K$^+$ flux into proteoliposomes reconstituted
with the partially purified fraction identified in lane 3 of Fig.
1B. K$^+$ transport was identified as electrophoretic by the fact
that it required CCCP when liposomes were suspended in KCl
medium (Fig. 3A) and occurred spontaneously in KSCN me-
dium (trace 1, Fig. 3B).

As shown in Fig. 3A, addition of CCCP induced rapid K$^+$
uptake from KCl medium, characteristic of a pathway for
electrophoretic K$^+$ flux. The electrical driving force for K$^+$
uptake was provided by a pH gradient of 2 units, so that $\Delta\Psi$
on upon addition of CCCP was about 120 mV. There was no K$^+$
flux in the absence of CCCP, confirming the low permeability
to Cl$^-$ ion and the absence of an electroneutral K$^+$/H$^+$ ex-
change pathway in the preparation. There was no K$^+$ flux in
the presence of CCCP when protein was omitted, reflecting
low permeability of liposomes to K$^+$. Further identifica-
tion of this transport with the mitochondrial K$^+$ channel was
provided by its inhibition by MgATP or glibenclamide (data
not shown).

As shown in traces 1–3 of Fig. 3B, rapid K$^+$ uptake from
KSCN medium occurred spontaneously, also characteristic of

Fig. 2. Electrical conductance in lipid bilayer membrane
reconstituted with purified K$^+$ channel. a, gel pattern of
the purified fraction, showing a predominant band at 54 kDa ("X"). b, molecular mass standards; and current recording following fraction-
ation of inner membranes from beef heart mitochondria and addition of 1 mg per ml of the partially purified fraction to the cis side of a chamber separated by a planar lipid bilayer. A voltage of 85 mV was
applied across the membrane to obtain the current recording. The
observed upward deflection of the signal reflects opening of a channel
carrying a current of about 2.55 pA.

Fig. 3. Electrophoretic K$^+$ uptake into proteoliposomes re-
constituted with the partially purified K$^+$ channel. Intravascular,
K$^+$, "[K$^+$]", is plotted versus time after addition of proteolip-
osomes to assay medium. The partially purified protein fraction from
Fig. 1b was reconstituted into liposomes containing the fluorescent
K$^+$ probe PBFI, and K$^+$ uptake was measured as described under
"Experimental Procedures." A, CCCP-induced K$^+$ uptake from KCl
medium. K$^+$ uptake was measured in the presence (+CCCP) or
absence (−CCCP) of 1 μM CCCP in the KCl assay medium described
under "Experimental Procedures." The pH gradient was 2, alkaline
inside. The requirement for CCCP demonstrates that K$^+$ transport
was electrophoretic. B, spontaneous K$^+$ uptake from KSCN medium.
K$^+$ uptake was measured in standard KSCN assay medium containing
0.5 mM EDTA, described under "Experimental Procedures," that was
modified as follows. Trace 1, no modification; trace 2, 3 mM Mg$^{2+}$, no
EDTA; trace 3, 200 μM ATP, added as Tris salt; trace 4, 200 μM ATP and
3 mM Mg$^{2+}$, no EDTA; trace 5, KCl substituted for KSCN. The
requirement for SCN$^-$ (compare traces 1 and 5) demonstrates that
K$^+$ transport was electrophoretic.

d a pathway for electrophoretic K$^+$ flux. The electrical driving
force for K$^+$ uptake, about 180 mV, was provided by the

Inhibition of K$^+$ Transport via the ATP-dependent K$^+$ Chan-
nel by ADP and ATP, and the Effects of Mg$^{2+}$ and Ca$^{2+}$—
Trace 4 of Fig. 3B shows the profound inhibitory effect of
ATP on K$^+$ transport via the reconstituted mitochondrial K$^+$
channel. Inhibition required both Mg$^{2+}$ and ATP. ATP had
no effect in the absence of Mg$^{2+}$ (trace 2), and Mg$^{2+}$ had no
effect in the absence of ATP (trace 3). The lack of effect of
Mg$^{2+}$ differs from conclusions drawn from studies in intact
mitochondria that the K$^+$ channel is inhibited by Mg$^{2+}$
(22, 23). The same effects were observed with 3 mM Ca$^{2+}$;
Ca$^{2+}$ alone had no effect, whereas Ca$^{2+}$ plus ATP inhibited
transport.

Fig. 4 contains dose-response curves from experiments to
investigate interactions between nucleotides and divalent cat-
ions. Fig. 4A contains dose-response curves for inhibition of
K$^+$ uniport by ATP and ADP in the presence of 3 mM Mg$^{2+}$.
When the inhibitors were added alone, $K_{[ATP]}$ was 39 μM,
Inhibition of K⁺ channel by glibenclamide at different [Mg²⁺]. The data are plotted as percentage inhibition of K⁺ uptake into proteoliposomes reconstituted with the K⁺ channel protein. Three separate preparations that exhibited normal ATP-dependent K⁺ transport activity were made parallel to reconstituted proteoliposomes containing the Na⁺ probe, SBFI, and assayed for Na⁺ transport. No Na⁺ transport was observed (not shown), and we conclude that the mitochondrial ATP-dependent K⁺ channel bears a strong resemblance to its counterparts in plasmalemma (9, 10, 24). To probe for other similarities, we examined the effects of the sulfonylurea, glibenclamide, which is a potent and classical inhibitor of plasmalemmal ATP-dependent K⁺ channels (25, 26). Curve A of Fig. 5 is a dose-response curve for glibenclamide inhibition of K⁺ uniport. Glibenclamide was capable of complete inhibition of K⁺ uniport in the reconstituted system, and its Kᵢ was 62 nM. This result is typical of five similar experiments which yielded Kᵢ values of 50–70 nM.

The data in Curve A of Fig. 5 were obtained in medium containing 0.5 mM EDTA and no added divalent cations. The data in Curves B and C of Fig. 5 were obtained in 0.5 and 3 mM Mg²⁺, respectively. The surprising result was that Mg²⁺, which itself had no effect on K⁺ uniport, reduced the inhibitory potency of glibenclamide. This result further supports the suggestion that Mg²⁺ ion may interact directly with the channel protein.

Inhibition of K⁺ Transport via the ATP-dependent K⁺ Channel by DCCD—Gauthier and Diwan (27) were the first to show that the alkylating agent DCCD partially inhibited K⁺ uniport in intact mitochondria. We found no K⁺ uniport activity in the fraction isolated from mitochondria that had been pretreated with 50 nmol of DCCD per mg of protein for 1 h on ice (data not shown), suggesting that the K⁺ channel had been irreversibly blocked by DCCD. This result is significant because the preincubation with DCCD was carried out without Mg²⁺ depletion, a condition in which the mitochondrial K⁺/H⁺ antiporter is not irreversibly bound by DCCD (28).

We next examined the effect of treating the proteoliposomes with DCCD. Fig. 6A contains traces from proteoliposomes that were untreated (trace 1) and treated (trace 2) with 1 mM DCCD for 3 h on ice. DCCD inhibition followed pseudo-first order kinetics, as shown in Fig. 6B.

Cation Selectivity of the Mitochondrial K⁺ Channel—On three separate preparations that exhibited normal ATP-dependent K⁺ transport activity, we made parallel proteoliposomes containing the Na⁺ probe, SBFI, and assayed for Na⁺ transport. No Na⁺ transport was observed (not shown), and we conclude that the mitochondrial ATP-dependent K⁺ chan-

![Fig. 4. Dose-response curves for inhibition of the ATP-dependent K⁺ channel by ATP and ADP (with Mg²⁺) and by Mg²⁺ and Ca²⁺ (with ATP). The data are plotted as percent inhibition of K⁺ uptake into proteoliposomes reconstituted with the K⁺ channel protein. Assays were carried out in standard KSCN medium, supplemented as described in the legend to Fig. 3. Initial rates of K⁺ uptake in millimolar/s were used. The rate in KSCN medium containing 0.5 mM EDTA was used for 0% inhibition, and the rate in the same medium in which KCl was substituted for KSCN was used for 100% inhibition. A, dose-response curves for inhibition by ATP (●), ADP (△), and ADP plus 50 μM ATP (○) in 3 mM Mg²⁺. Linear regression of Hill plots of these data (not shown) yielded Hill slopes of 1.1 to 1.20 and Kᵢ values of 39 μM for ATP, 280 μM for ADP, and 639 μM for ADP in the presence of 50 μM ATP. Four separate reconstitution experiments yielded an average Kᵢ of 43 μM for ATP in the presence of 3 mM Mg²⁺. B, dose-response curves for Mg²⁺ and Ca²⁺ inhibition in 0.5 mM ATP. The Kᵢ for Mg²⁺ (△) was 80 μM, and the Kᵢ for Ca²⁺ (○) was 151 μM. The solid curve was plotted using these values. The solid curves used a Hill slope of 1. [Mg²⁺] and [Ca²⁺] are expressed as free concentrations in the assay media. This result was typical of three separate experiments with Mg²⁺ and two with Ca²⁺.

and Kᵢ[ADP] was 280 μM. These values were reproducible ± 15% in six and two experiments, respectively. The right dose-response curve in Fig. 4A reports inhibition by ADP in the presence of 50 μM ATP. The observed Kᵢ[ADP] was 639 μM. This is entirely consistent with competition between ATP and ADP for the same site, in which Kᵢ[ADP] = Kᵢ[ADP] (1 + [ATP]/Kᵢ[ATP]). Using this relationship to calculate Kᵢ[ADP] in the presence of 50 μM ATP also yields 639 μM.

Fig. 4B contains a dose-response curve for inhibition of K⁺ uniport by Mg²⁺ and Ca²⁺ in the presence of 0.5 mM ATP. The Kᵢ values, referring to free [Mg²⁺], were 80 μM for Mg²⁺ and 151 μM for Ca²⁺, and these values were reproducible ± 15% in four and two experiments, respectively. In these protocols, Mg²⁺ and ATP were added to the assay medium prior to addition of proteoliposomes. When proteoliposomes were preincubated with 1 mM Mg²⁺, then diluted 100-fold into the assay medium, 100 μM ATP inhibited more than 50% despite the fact that total [Mg²⁺] was only 10 μM. There was no inhibition in the absence of ATP. This effect, which is being investigated further, suggests that Mg²⁺ and ATP interact independently with the ATP-dependent K⁺ channel.

Inhibition of K⁺ Transport via the ATP-dependent K⁺ Channel by Glibenclamide and the Effect of Mg²⁺ on Glibenclamide Inhibition—In its high sensitivity to ATP, the mitochondrial
Reconstitution of Mitochondrial K⁺ Channel

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Inhibition of the ATP-dependent K⁺ channel by DCCD. K⁺ uptake was measured after addition of proteoliposomes to standard KSCN assay medium. A, inhibition of K⁺ uptake by DCCD. Intravesicular K⁺, [K⁺], is plotted versus time after addition of proteoliposomes to assay medium. — DCCD, control proteoliposomes were preincubated for 180 min at ice temperature with 1% ethanol. + DCCD, proteoliposomes were preincubated for 180 min at ice temperature with 1 mM DCCD in ethanol. B, pseudo-first order kinetics of DCCD inhibition of K⁺ uptake. The log of relative rate (rate in DCCD-treated proteoliposomes divided by rate in ethanol control) is plotted versus the duration of exposure to DCCD. Initial K⁺ uptake rates from experiments such as those plotted in A were obtained following incubation with DCCD and ethanol (control) for varying times.

Dependence of K⁺ transport through the ATP-dependent K⁺ channel on [K⁺]. Rate of K⁺ uptake into liposomes reconstituted with the partially purified K⁺ channel is plotted versus medium K⁺ concentration, [K⁺]. Medium was the same as standard KSCN medium except that the permeant Cl⁻ was substituted for SCN⁻. Mixtures of KClO₄ and TEAClO₄ were used to vary [K⁺]. Nonlinear regression analysis yielded a Kₘ value of 32 mM. A similar value was obtained from a double reciprocal plot of the same data (not shown).

Dependence of K⁺ transport through the ATP-dependent K⁺ channel on pH and ΔΨ. The ATP-dependent K⁺ channel exhibited little dependence on pH, as shown in Fig. 8. In contrast, K⁺ transport was highly dependent on ΔΨ, as shown in Fig. 9. Theory predicts that flux through a non-voltage-gated channel should be proportional to e⁻βv at high ΔΨ, where u = FΔΨ/room temperature, and β is the fractional distance to the first energy barrier peak (29). For ion leak, the barrier is near the center of the bilayer, and β is ~0.5. The log-linear plot of K⁺ flux versus u (Fig. 9B) yields a β value of ~0.25, consistent with a channel containing a single energy well near the center of the membrane. Because the role of voltage in the driving force can account entirely for the observed voltage dependence, there is no indication that the mitochondrial ATP-dependent K⁺ channel is voltage gated.

Dependence of K⁺ transport through the ATP-dependent K⁺ channel on ΔΨ. A, rate of K⁺ uptake into liposomes reconstituted with the partially purified K⁺ channel is plotted versus medium pH. ■, 150 mM KSCN medium; ○, 150 mM KClO₄ medium. Internal pH was maintained at 7.4. External medium was adjusted to indicated pH.

Dependence of K⁺ transport through the ATP-dependent K⁺ channel on ΔΨ. B, ln(K⁺ uptake rate) is plotted versus the reduced voltage, u, where u = FΔΨ/room temperature. Mixtures of 150 mM KSCN and 150 mM KClO₄ were used to vary ΔΨ, which was calculated from the ratio [SCN⁻]/[SCN⁻]. Linear regression of the data in B yielded a slope (β, see text) of 0.24.
Reconstitution of Mitochondrial K⁺ Channel

Volume-regulating K⁺ Cycle in Mitochondria—The chemiosmotic mechanism of energy coupling (30) imposes a severe burden on mitochondrial volume homeostasis. Electrogenic proton ejection results in a very high ∆Ψ, which will tend to cause excessive uptake of cations, especially potassium, from the cytosol. Recognizing this consequence, and revealing a deep physiological insight, Mitchell (30) proposed electroneutral exchange carriers to permit expulsion of cations from the matrix. Subsequently, Brierley and co-workers (31, 32) proposed that mitochondrial K⁺ homeostasis is maintained by a regulated interplay between an electrophoretic cation entry mechanism and an exchanger-mediated efflux pathway.

Fig. 10 describes a complete K⁺ cycle in mitochondria. The gradients operating in vivo are such that electrically coupled K⁺/H⁺ exchange and electroneutral K⁺/H⁺ exchange move K⁺ across the membrane in opposite directions. Because each mode of K⁺/H⁺ exchange leads to transport of K⁺ salts, due to simultaneous operation of the electroneutral anion exchangers and because the inner membrane is highly permeable to water, net transport of K⁺ salts in either direction will cause volume changes. Thus, the physiological function of this futile cycle is to regulate mitochondrial volume (28). To maintain constant volume requires a net K⁺ flux of zero, and this can only be achieved if K⁺ efflux via electroneutral K⁺/H⁺ exchange precisely balances K⁺ uptake via electrophoretic K⁺ uniport, and vice versa.

The Electroneutral Potassium Efflux Pathway in Mitochondria—K⁺ efflux is regulated by a “carrier brake mechanism” (33) in which volume-sensing inhibition of the K⁺/H⁺ antiporter is provided by variations in free matrix Mg²⁺ and H⁺ ions. As K⁺ enters the matrix in association with anions and water, matrix [Mg²⁺] and [H⁺] will fall, and the carrier is released to expel the extra K⁺. The carrier brake mechanism is well designed to adjust K⁺/H⁺ exchange rates to fluctuations in K⁺ uptake via K⁺ uniport.

The K⁺/H⁺ antiporter is irreversibly inhibited by DCCD, but only when the carrier is in an active state, free of reversible inhibitors such as Mg²⁺, H⁺, and amphiphilic amines (28). DCCD inhibition enabled us to identify the carrier as an 82-kDa protein (34), and this protein has been purified to homogeneity with reconstitutive activity (35).

Electrophoretic Potassium Uptake Pathways in Mitochondria—Gamble (36, 37) first showed that 4K⁺/K⁺ exchange, which is very low in nonrespiring mitochondria, is stimulated by respiration. Other workers have also observed physiological and pathological states in which mitochondrial K⁺ transport is stimulated (38–45). Whereas these studies were suggestive, a decisive conclusion that mitochondria have the ability to activate K⁺ uniport could not be established. The primary reason for this uncertainty is the obligatory coexistence of considerable K⁺ leak in experiments on K⁺ uniport in intact mitochondria. At the high potentials normally used to study K⁺ uniport, ion leak is exponential with ∆Ψ (29). Accordingly, K⁺ uptake is exquisitely sensitive to variations in ∆Ψ, and attempts to demonstrate a component due to a specific K⁺ uniporter were inconclusive. It became necessary to demonstrate reconstitution of a specific K⁺ uniport process, and this effort was undertaken by several laboratories.

K⁺ uniport following reconstitution was first observed by Mironova et al. (2) and subsequently by Diwan and coworkers (3). Characterization of reconstituted K⁺ transport has been limited. In particular, there is no previous evidence that reconstituted K⁺ transport is inhibited by ATP and glibenclamide.

Several laboratories have attempted to identify K⁺ uniport with a specific inner membrane protein. Mironova et al. (2) isolated a fraction from beef heart mitochondria that induced monovalent cation-selective conductivity channels in bilayer lipid membranes and identified activity with a 54–57-kDa protein. Diwan et al. (3) demonstrated K⁺ uniport activity of a protein mixture obtained from a quinine affinity column which also induced conductance in the bilayer (4), and they attributed activity to a 53-kDa protein. Diwan et al. (46) subsequently found that the predominant 53-kDa protein was aldehyde dehydrogenase. Diwan’s laboratory (47) has now subfractionated this mixture. Patch clamp analysis has led to identification of a 57-kDa protein with an ion channel conductance of 40 pS that transports Na⁺ and K⁺ equally. The fraction in which the 53-kDa band was predominant was found to have a conductance of 130 pS.

Evidence for an ATP-dependent K⁺ Channel in Mitochondria—Our results, which represent over 2 years of study of this process, establish that the mitochondrial inner membrane contains an ATP-dependent K⁺ channel. The channel has been highly purified; however, activity cannot yet be identified with a specific inner membrane protein. We are investigating the amino acid sequence of the 54-kDa protein identified in Fig. 1B, which is the major component of the purified fraction, and we are attempting to subfractionate these proteins using ATP-agarose affinity columns. Because this channel is highly active, it is likely to be of low abundance, creating difficulties for purification, identification, and recovery.

Recently, ATP-dependent potassium channels have been identified by direct patch clamping of fused giant mitoplasts from rat liver mitochondria (8). Inoue et al. (8) observed ATP inhibition (I₅₀ ~ 800 μM) in the absence of added Mg²⁺, and higher levels of glibenclamide (5 μM) were required to inhibit the channel than were required in our studies (K₅₀ ~ 50 nM). These discrepancies may be due to the presence of 0.55 mM Ca²⁺ (10 nM free Ca²⁺) in the assay medium used by Inoue et al. (8) and to the pretreatment with high levels of Ca²⁺ during vesicle fusion. Our preliminary studies indicate that prior exposure to high levels of divalent cations increases the apparent affinity of the ATP-dependent K⁺ channel for these cations, suggesting a slow dissociation constant.

Most of the results we have reported were obtained from the rat liver mitochondrial preparation, but results with the beef heart mitochondrial preparation were qualitatively indistinguishable with respect to K⁺ specificity and inhibition with high affinity by ATP and glibenclamide. We have not yet carried out detailed kinetic studies on the fraction from beef heart mitochondria.

The Physiological Role of the Mitochondrial ATP-dependent K⁺ Channel—Electrophoretic K⁺ uptake will dissipate energy and uncouple oxidative phosphorylation (see Fig. 10); therefore the mitochondrial K⁺ channel must perform be regulated.

**Fig. 10. Mitochondrial K⁺ cycle.** Electrogenic H⁺ ejection by the mitochondrial redox chain drives electrophoretic K⁺ uptake into the matrix by parallel leak and channel pathways. Internal K⁺ is then released in exchange for protons via the electroneutral K⁺/H⁺ antiporter, which is dynamically regulated by matrix free Mg²⁺ ions. The K⁺ cycle is a futile cycle required for physiological control of matrix volume.
The observed high affinity for ATP meets this expectation that the K⁺ channel should normally be closed but raises the obverse question, how can it be opened under physiological conditions? This is a subject of active investigation in our laboratory, and we hypothesize that an unknown activator, perhaps another nucleotide, reduces channel affinity for ATP.

The physiological mission of the mitochondrial K⁺ channel is unknown, but two possibilities suggest themselves. (i) The K⁺ channel may open up to expand matrix volume during mitochondrial biogenesis and in association with synthesis of proteins and expansion of membranes. Volume expansion would require net K⁺ uptake, mediated by an imbalance between activity of the ATP-dependent K⁺ channel and the K⁺/H⁺ antiporter. (ii) The K⁺ channel and the K⁺/H⁺ antiporter may increase their activities simultaneously in order to dissipate energy. Regulated energy dissipation during periods of low cellular requirements for ATP would be a useful mechanism for providing heat to the cell as well as preventing obesity.

Relationships between Mitochondrial and Plasmalemmal ATP-dependent K⁺ Channels—ATP-sensitive K⁺ channels, identified by patch clamp studies, have been found in plasma membranes of cardiac and skeletal muscle, smooth muscle (10), neurons (9), and pancreatic β-cells (48). The salient properties of all K⁺ATP channels are selective K⁺ conductance, inhibition by ATP with unusually high affinity, regulation of ATP inhibition by other nucleotides, and inhibition by glibenclamide with high affinity. In pancreatic β cells, K⁺ATP channels have a physiological role in glucose-stimulated insulin secretion. Channel opening blocks insulin secretion, whereas channel blockade stimulates insulin secretion (49). In heart, glycolysis has been suggested as a preferential source of ATP for regulating the K⁺/ATP channels (50). K⁺/ATP channels are thought to be responsible for cellular K⁺ loss during cardiac ischemia and hypoxia (51).

ATP inhibits K⁺ATP channels by decreasing opening frequency (48). Free ATP mediates channel closure in most cases (49), but MgATP is a potent inhibitor of K⁺ATP channels in cardiac myocytes. Other nucleotides and their nonhydrolyzable analogs have various effects on K⁺ATP channel activity. In cardiac myocytes, ADP and GDP stimulate channel activity in the presence of ATP and Mg²⁺, and an emerging opinion is that plasma membrane K⁺ATP channels have two different nucleotide binding sites, one mediating activation and the other inhibition (24).

Despite numerous attempts, reconstitution of functionally active K⁺ flux that is inhibited by ATP and glibenclamide has not been achieved with plasmalemmal K⁺ATP channels, nor has the plasmalemmal protein responsible for K⁺ATP channel activity been identified with certainty (see Refs. 52–54). This field is likely to advance rapidly in view of the fact that the renal epithelial K⁺ channel from thick ascending limb has been cloned and sequenced by Drs. Kevin Ho and Steven Hebert of Brigham and Women's Hospital, Boston. The cDNA encodes a 391-amino acid protein containing an ATP-binding domain and a conserved pore motif that is similar to that found in voltage-regulated K⁺ channels.³

The K⁺ for ATP inhibition of plasma membrane K⁺ATP channels varies among heart (Kᵢ = 25–500 μM), skeletal muscle (Kᵢ = 17–135 μM), and pancreatic β cells (Kᵢ = 10–70 μM) (10). These values may be compared to 45 μM in mitochondria (this report). It seems evident that the mitochondrial K⁺ATP channel differs no more in its properties from the plasmalemmal K⁺ATP channels than the latter differ among themselves. Furthermore, as described in this report, it exhibits all the salient properties characteristic of this functional family: it conducts K⁺ ions selectively, it is inhibited by ATP with unusually high affinity, and it is inhibited by glibenclamide with high affinity. It seems likely that the mitochondrial and plasmalemmal K⁺ATP channels belong to the same gene family.

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Reconstitution of Mitochondrial K⁺ Channel